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# Chemosensitisation Effect of Verapamil and Cyclosporin A *in vitro* is Reduced under Acidic pH Conditions

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MULTIDRUG RESISTANCE (MDR) seems to contribute to the insensitivity to chemotherapy in haematologic malignancies and solid tumours [1]. This decreased intracellular drug accumulation is due to the drug transport activity of the MDR-1 gene-encoded P-glycoprotein (P-gp) and can be inhibited by chemosensitisers or chemomodulators such as verapamil (VPM), cyclosporine A (CsA), tamoxifen (TMX) and other drugs [2]. In phase I and II clinical resistance modifier trials with VPM and CsA, low response rates were observed in patients with advanced solid tumours, with transient and partial anti-tumour effects at maximum, whilst the results obtained in drug-refractory haematological malignancies look much more promising [1]. This difference may be explained by the presence of multiple mechanisms of resistance in solid tumours and the limited accessability of the tumour compartment with its specific microenvironmental conditions. The functional vasculature in solid tumours decreases during their growth and leads to increased hypoxia, anaerobic metabolism and acidosis [3]. The pH of the extracellular fluid ($pH_0$) has been measured in tumours by insertion of small diameter pH electrodes and found to be shifted toward more acidic values for larger tumours than that measured in normal tissues (median pH value approximately 6.9–7.0 vs. 7.4–7.5). The corresponding intracellular tumour pH values ($pH_i$), as determined by $^{31}P$ nuclear magnetic resonance spectroscopy were near normal [4]. Low $pH_0$ values have been demonstrated to reduce significantly the intracellular accumulation and toxicity of anthracyclines [5].

In this study we have investigated the effects of low $pH_0$ conditions on the chemosensitising (MDR-reversing) activity of VPM and CsA in doxorubicin sensitivity assays using two colon cancer-derived cell lines, HT-29 and SW 480 (ATCC, Rockville, Maryland, U.S.A.; HTB-38 and CCL 228) as target cells. These cells express low levels of P-gp, typical of drug-refractory solid tumours *in vivo* [1]. $IC_{50}$ values were measured in tissue culture medium and VPM (10 μmol/l) or CsA (1 μg/ml) supplemented medium with $5 \times 10^4$ cells/well of microtitre plates, 2-fold dilutions of doxorubicin and labelling with [$^3$H]thymidine (37 kBq/well) for the last 18 h of the 48-h incubation period.

The results demonstrate the reduced toxicity of doxorubicin at lower pH values for both cell lines (5–7-fold) and reduction of the chemosensitising effects of both VPM and CsA for pH values < 7.20 (20–45% reduction; $P < 0.05$ for pH = 6.83 and 7.20 vs. pH = 7.40–7.67) with a marked increase of the MDR modulatory effect of VPM in the alkaline pH range (Table 1).

*Table 1. Doxorubicin sensitivity, MDR reversal by VPM/CsA and VPM binding capacity of two colon carcinoma cell lines at different extracellular* pH *conditions*

| | $pH_0$ 6.83 | 7.20 | 7.40 | 7.57 | 7.67 |
|---|---|---|---|---|---|
| HT-29/doxorubicin | | | | | |
| $IC_{50}$ (ng/ml) | 210.6 | 62.5 | 30 | 33 | 45 |
| $R_{VPM}$ | 1.8 | 2.15 | 2.3 | 2.6 | 6.8 |
| $R_{CsA}$ | 0.8 | 0.9 | 1.25 | 2.5 | 3.4 |
| SW-420/doxorubicin | | | | | |
| $IC_{50}$ (ng/ml) | 78 | 38 | 16 | 24 | 53 |
| $R_{VPM}$ | 2.1 | 1.8 | 2.7 | 7.7 | 37.5 |
| $R_{CsA}$ | 1.3 | 1.5 | 2.3 | 2.2 | 4.2 |
| VPM-binding | | | | | |
| % bound | 47±3.8 | 80±4.0 | 100% | 140±12 | 170±19 |

Reversal (R): $IC_{50}$ in medium/$IC_{50}$ in the presence of the chemosensitiser.

The binding of *N*-methyl-$^3$H-verapamil (NEN, Boston, Massachusetts, U.S.A.) to five different colon carcinoma cell lines (including CaCo-2, HCT-8 and SW-620) was measured in 10% bovine serum albumin/Hepes-buffered Ringers solutions, titrated with *N*-methylglucamine to pH values of 6.6, 7.0, 7.4 and 7.8, respectively. For each pH $1 \times 10^6$ cells were incubated with 74 kBq verapamil for 18 h at room temperature, cell-bound radioactivity counted after washing and the values obtained for the solution with pH = 7.4 set to 100% binding ($3–6 \times 10^4$ cpm; Table 1, bottom). The cellular uptake of VPM increases significantly with increasing extracellular pH, showing good correlation with the parallel increases in MDR reversal (correlation coefficient $r = 0.8$ for both cell lines).

In conclusion, acidic extracellular conditions, similar to *in vivo* tumour microenvironments, reduce not only the cytotoxicity of anthracyclines, but also the chemosensitising effects of VPM and CsA, most likely contributing thereby to the low response rates observed in resistance modifier trials in solid tumours. In the case of VPM, the main cause of the varying MDR reversal seems to be related to the pH-dependent cellular uptake. Chemomodulators should be tested under more representative conditions (tumour minimal pH values as low as 6.0) in binding and MDR reversal assays and alkalinisation of tumour tissues may be investigated for the improval of the P-gp-directed antitumour therapy.

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